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0EFFECT OF HONEY BEE PROPOLIS ON THE KEEPING QUALITY OF LABNEH MADE FROM BUFFALO،S MILK

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ABSTRACT

Three yeast cultures namely *Saccharomyces cerevisia, Debaryomyces hansenii* and *Kluyveromyces marxianus* were tested for their resistance to propolis extract.

Propolis aqueous extract (10% w/v) at the ratios 0.25, 0.50, 0.75 and 1.00% was examined. Changes in counts of yeasts were monitored after 7 days at 25°C using synthetic medium. The effect of adding aqueous extract of propolis on the microbiological, chemical properties and organoleptic quality attributes of Labneh was investigated. Labneh was made from buffalo milk (5.5% fat) using 2% of mixed starter culture. The product was packaged in PVC (250g), stored at 6ºC for 3 weeks and analyzed weekly for chemical composition, microbial and organoleptic properties.

The presence of 0.75 and 1.00% proplis was the most antimycotic for the tested yeast, while propolis 0.25% stimulated growth of the tested yeasts. No marked differences were recorded in total solids, fat and protein contents between the control and treated samples. The chemical composition of Labneh from different treatments was affected slightly during cold storage. However, the reduction in pH, and development of diacetyl and Acetaldehyde were the highest in control, but lowest in Labneh with the highest propolis concentration. Higher propolis concentration proved to be effective in decreasing yeast, mould, lactic acid bacteria and total plate counts. Although the addition of 1.00% propolis could prolong the shelf life to to 21 days without any sings of yeast spoilage, but the Labneh was unaccepted according to its very sharp flavour and brownish color. It is recommended to add propolis (10% w/v) aqueous solution at the ratio of 0.25 and 0.50% to Labneh to prolong the shelf life and high scour sensory evaluation.

INTRODUCTION

Concentrated yogurt, known as Labneh in the Middle East, is widely consumed, chiefly as a sandwich spread, in the Middle East and Balkan regions (Tamime *et al.,* 1989). Labneh is produced by removing a proporation of the whey from cow،s milk yogurt until fat and total solids contents of 9 to 11 and 23 to 25% are attained, respectively (Tamime and Robinson, 1999). Labneh is white to creamy paste that has a smooth texture with a taste crossing between sour cream and cottage cheese and characteristic sharp flavor that is largely modulated by diacetyl produced during fermentation (Varnam and Sutherland, 1994; Al-Kadamany, *et al.,* 2002). The product is packaged and stored in refrigerator for sale and consumption. The high concentration of lactic acid and limited access of air during refrigerated storage are best conditions for the product when general good manufacturing are not fully followed.

The presence of live starter bacteria and yeast and mold contaminants coupled with packaging/storage conditions lead to the formation of off-flavors and other undesirable physico-chemical changes that eventually lead to product failure (Muir and Banks, 2000). The stated shelf life of cloth-bag

Labenh, produced by major dairy processors, is between 14 and 21 d and is largely based on commercial experience, the behavior of starter cultures (Ozer and Robinson ,1999) , yeast microflora (Yamani and Abu-Jaber , 1994). These yeasts can cause spoilage and may adversely affect public health (Waiker and Ayers, 1970; Suriyarachchi and fleet, 1981; Todd, 1983 and Taylor, 1980). Yamani and Abu-Jaber (1994) mentioned that the major yeast responsible for the spoilage of labneh were: *Saccharomyces cerevisiae, Tricosporn brossicae, Creyptococcus curvates, Kluyveromyces marxianus, Tricosporn cutaneum, Debaryomyces hansenii, Pichia farinose, Geotrichum candidum* and *candida blankii..*

Many investigators (Dagher and Ali 1985, Tamime and Crawford 1984 and Ghadeer et al., 1997) used different concentrations of H_2O_2, potassium sorbate and sodium benzoate as a mean of prolonging the shelf- life of Labneh by reducing the total count and yeast and molds. But, according to the potential harmfulness of these artificial food preservatives and high demands of consumers for the safety of food, it became necessary to develop some natural preservatives to replace these artificial hazardous preservatives.

Propolis is a natural product collected by honey bees workers for different purposes. It has antimicrobial, antifungal, antiviral, immunostimulant and antioxidant properties (Bratter *et al.,* 1999 and Koo *et al.,* 2000).

The strong antimicrobial activity of propolis is due to its flavonoids contents (Grange and Devey 1990). At least 38 flavonoids have been found in propolis (Greenaway *et al.,* 1990). Propolis had shown an inhibitory effect on a variety of microorganisms (Kujungiev *et al.,* 1999). Moreover, propolis is also used in human nutrition due to its contents of amino acids and vitamins A, B_1, B_2, B_6, C and D (Ghisalberti 1979). Propolis is a stable product, that retain antimicrobial activity even when stored for one year long. It can be used as a preservative in food products due to its antioxidant and antimicrobial activities.

In Egypt, Moawad *et al.,* (2001a , 2001b), Dabiza (2006) and Moawad *et al.,* (2002) sprayed propolis on the surfaces of Ras cheese and ultrafiltered soft cheese, which protect completely this surfaces against mould and bacterial growth.

The present paper examined the effect of the natural preservative "propolis" against the most yeast responsible for Labneh spoilage namely, *K. morxianus, D. hasenii* and *S. cerevisiae*, and the compositional quality, microbiological parameters and sensory evaluation of Labneh made from buffalo's milk.

MATERIALS AND METHODS

Propolis samples were collected from the hyprid honey bee colonies at Fayoum Governorate, Egypt. Samples were collected by scraping the small pieces of propolis. Collected samples were weighed and stored separately in the refrigerator until used as the method described by Muszynsks *et al.,(*1993).

Buffalo's milk was obtained from the dairy processing plant of Food Tech. Res. Institute. The fat content and casein/fat ratio were standardized to be 5.5% and 0.7, respectively.

Three yeast cultures responsible for the spoilage of labneh were obtained from the Faculty of Agric. Ain Shames Univ., The cultures were: *Saccharomyces cerevisia, Debaryomyces hansenii* and *Kluyveromyces marxianus.*

Glucose peptone yeast extracts medium (GPY.) containing 2% glucose, 0.5% peptone. 0.5% yeast extract and 1.4% agar was used for activation and enumeration.

Freeze dried mixed starter culture (*Lactobacillus delbrueckia ssp. bulgaricus* and *Streptococcus salivrius ssp. thermophilus* (1:1) was obtained from Chr. Hansenii Lab. Oritorun A/S Copenhagen Denmark.

Activation of yeast cultures was repeated three times on GPY agar, with incubation for 3d. at 25^0C in order to activate the yeasts. Pure activated colonies were maintained on GPY agar slants and stored at 5^0C.

The particular yeast was grown on GPY agar slants at 25^0C for ten days. The GPY agar slants was washed using 100 ml sterile .005% tween 80. A Yeast count of culture was detected using the pour plate technique with GPY agar to which 100 mg chloromphenicole were added (Frank *et al.,* 1985). Colonies were counted after 3d. of induction at 25^0C.

The antimycotic activity of propolis against yeast was evaluated by adding the propolis to 50 ml of GPY broth for final concentrations of 0.00, 0.10, 0.25, 0.50 and 1.00% w/v. The flasks containing propolis and GPY medium were autoclaved at 121^0C for 15 min. cooled at room temp., inoculated with 1.0ml of previous inocula (~10^4cfu/ml) and incubated at 25^0C for 7 days. The yeast colonies of each flask were counted using the pour plate technique with GPY agar to which choromphenicol was added.

A stock 10% (w/v) propolis aqueous solution was prepared as follows: propolis pieces were grounded soaked in distilled water (1:10 w/v) for 7 days with periodic shaking. The extract was filtered through Ziess filter to obtain sterile aqueous propolis solution (10%). Aliquot of 2.5, 5 and 7.5 ml/liter milk (w/v), were added from stock solution. (Moawed *et al.,* 2001b).

Cloth-bag Labneh, produced as described by Yamani and Abu-Jaber (1994). The product was made by heating buffalo's milk to 85^0C for 20 min, colling to 40^0C, incoculating with 2% starter culture (*Lactobacillus delbrueckie ssp bulgaricus* and *Streptococcus salivrius ssp thermophilus* 1:1) and holding for 4h until a pH 4.6 was attained. The resulting yogurt was placed in cloth bags and left to drain by gravity at 6^0C overnight. The obtained Labneh was divided into five equal parts. The first part with no additives served as a control. For the other four portions, 0.25, 0.50, 0.75 and 1.00% of propolis extract were added. The Labneh was packaged in PVC containers (250 g), and stored at refrigerator (6˚C) and analyzed when fresh and after 1, 2 and 3 weeks of storage. Three replicates were made from each treatment.

Total solids, total protein, fat and ash contents were determined according to the methods described by AOAC (1994). pH value was measured using a laboratory pH meter with glass electrode. Acetaldehyde

and diacetyl of the resultant Labneh were measured using Shimadzu (240-UV-Vis) Spectrophotometer (Japan) as described by Lee and Jago(1970).

Samples were microbiologically examined for total plate count and counts of mould and yeast according to APHA (1992), and Lactic acid bacteria were detected according to Samona and Robinson (1991).

Labneh samples were scored for organoleptic properties by a regular taste panel from 20 staff members of Dairy Department in Food Tech. Res. Inst. Labneh samples were evaluated for sensory attributes according to Keating and White (1990), Using a scheme of 15 points for appearance, 10 points for acidity, 30 points for body and texture and 45 points for flavour.

The result of chemical analysis and sensory evaluation were statistically evaluated by statistical Analysis System (SAS, 1994) software programs. Significance amange means was carried out using Duncan's multiple test at $p<0.05$.

RESULTS AND DISCUSSIONS

Evaluation of antimycotic activity of propolis in synthetic medium:

Data presented in Table (1) revealed the effect of adding different levels of propolis on yeast count in synthetic medium. Results clearly indicate the effect of the addition of propolis extract on the tested yeasts. It prevented *K. marxianus, D. hansenii* and *S. cerevisiae* at 1.00%. While propolis at 0.25% exhibited a stimulated effect to the tested yeasts. The results are in agreement with those of Abou Dawood (2002), who reported that the sensitivity of yeast flora of Labneh to spices.

Table (1): Effect of adding of different levels of propolis on yeast count (log 10 cfu/g) in synthetic medium after 7 days of incubation at 25°C.

Propolis Concentration %	Yeasts count (Log 10 cfu/g)		
	Saccharomyces cerevisiae	Debaryomyces hansenii	Kluyveromyces cerevisiae
0.00	5.98	6.42	5.83
0.25	5.24	5.89	5.21
0.50	3.82	4.15	3.28
0.75	1.21	1.31	-
1.00	-	-	-

Chemical analysis of Labneh:

It is obvious that the addition of propolis extract had a negligible effect on on the total solids of the resultant Labneh, while a slight increase in ash content was observed. The slight differences might be due to the increase of the amount of propolis concentration Table (2). These results are in agreement with those reported by Zedan *et al.,* (2006). The chemical composition of all treatments was affected slightly during cold storage periods.

Results clearly indicate that, the pH was affected with the addition of propolis extract (Table 2). There were marked differences in pH values

between Labneh from different treatments. During storage, Labneh made without additive (control) showed lower pH than those made with added propolis extract being negatively related to propolis concentrations (Zedan *et al.,* 2006). This might be due to the effect of propolis on the acid producing microorganisms (Moawad *et al.,* 2001a). The acidity increased with storage, due to the action of microorganisms in metabolizing milk components, particularly lactose and citrate into organic acids. Similar results were reported by El-Senaitly (1999).

With regards to acetaldehyde and diacetyl, data presented in Fig (1) showed that acetaldehyde and diacetyl contents were higher in control than all treatments. During cold storage period, acetaldehyde content of Labneh samples decreased, while diacetyl content increased. This might be due to the slow reduction of diacetyl to acetoin (Driessen and Puhan 1988).

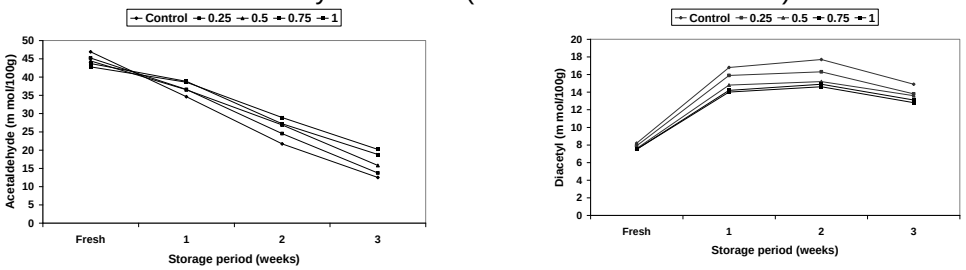

Fig (1): Effect of addition of different levels of propolis on the acetaldehyde and diacetyl contents of Labneh during storage period.

Table (2): Chemical composition of fresh and stored Labneh with and without the addition of propolis.

Gross Composition %	Storage Period (weeks	Control	Propolis concentration % 0.25	0.50	0.75	1.00	LSD
Total Solids	Fresh	28.67^{b}	28.32^{c}	28.12^{d}	27.91^{e}	27.67^{f}	0.1946
	1	28.96^{ab}	28.48^{bc}	28.35^{c}	28.02^{e}	27.85^{ef}	
	2	29.08^{a}	28.59^{bc}	28.61^{b}	28.15^{d}	28.03^{e}	
	3	29.17^{a}	28.93^{ab}	28.72^{b}	28.31^{c}	28.12^{d}	
Fat	Fresh	11.1	10.9	10.6	10.6	10.5	Ns
	1	11.3	11.1	10.9	10.8	10.7	
	2	11.3	11.2	11.1	10.9	10.8	
	3	11.4	11.3	11.2	11.1	11.0	
Protein	Fresh	13.97^{f}	13.81^{fg}	13.73^{g}	13.65^{g}	13.56h	0.2471
	1	14.52^{b}	14.32^{c}	14.02^{ef}	13.93^{f}	13.82^{f}	
	2	14.63^{ab}	14.51^{b}	14.23^{d}	14.15^{e}	14.03^{ef}	
	3	14.74^{a}	14.62^{ab}	14.44^{bc}	14.26^{cd}	14.15^{e}	
Ash	Fresh	1.21^{b}	1.17^{c}	1.15^{d}	1.13^{de}	1.11^{e}	0.0893
	1	1.23^{b}	1.19^{c}	1.20^{bc}	1.16^{cd}	1.14^{d}	
	2	1.28^{a}	1.22^{b}	1.21^{b}	1.18^{c}	1.16^{cd}	
	3	1.31^{a}	1.26^{ab}	1.23^{b}	1.21^{b}	1.19^{c}	
pH	Fresh	4.85^{cd}	4.93^{c}	5.12^{bc}	5.23^{a}	5.27^{a}	0.1805
	1	4.62^{e}	4.86^{cd}	4.98^{c}	5.17^{b}	5.19^{b}	
	2	3.95^{f}	4.57^{e}	4.74^{d}	4.83^{cd}	4.97^{c}	
	3	3.82^{f}	4.38^{g}	4.67^{de}	4.73^{d}	4.92^{c}	

Significant at 0.05 level.

Acetaldehyde content gradually decreased during the storage, presumably due to the demonstrated ability of numerous lactic organisms to reduce the acetaldehyde to ethanol or oxidize it to acetic acid (Essawy *et al.,* 2005).

Microbiological analysis of Labneh;

The effect of propolis on mold and yeast count was more pronounced Fig (2a). Reduction was dramatic in Labneh treated with high propolis concentration. Lower propolis concentration proportionally according to its concentration. Control Labneh showed continuous increase in count during the whole period of storage with visible growth after 4 days. This results substantiated the use of propolis as antifungal agent for protecting Labneh during its storage period. These results are similar to those reported by Abd El-Hady (2002) and Dobiza (2006), who reported that propolis has a known antifungal activity.

As indicated in Fig (2 b, c), the propolis extract used in Labneh processing had a remarkable effect on its total plate counts and Lactic acid bacteria counts. The count was reduced in all Labneh sample with propolis in different concentrations, the higher the propolis concentration the lower the bacterial count. This might be due to its destructive effect on some bacterial population and its inhibitory effect on some other bacteria (Simuth *et al.,* 1986).

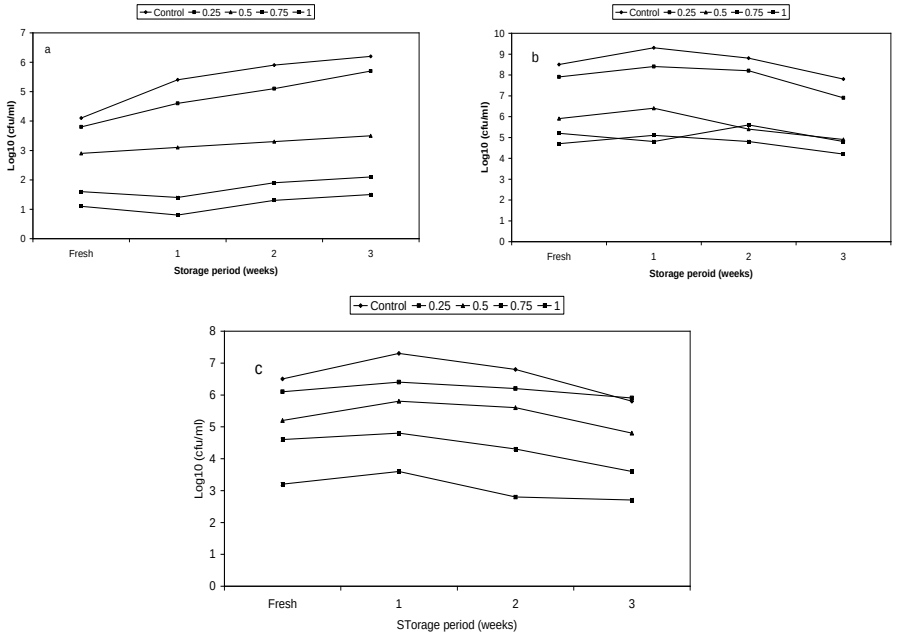

Fig (2): Changes in yeast and mould (a), total plate count (b) and lactic acid bacteria during storage of Labneh with and without the addition of propolis.

Sensory evaluation:

Results given in Table (3) revealed that Labneh treated with 0.25% and 0.50% propolis was the best. It is characterized by uniform texture, clean acid

flavour and accepted sharp flavour. It can be kept under refrigerated storage 6^0C for 21 days with high accepted flavour without sings of yeast spoilage. Although Labneh treated with 1.00% propolis could prolong its anticipated to 21 days without any sings of yeast spoilage, the Labneh was unaccepted according to its very sharp flavour and brownish color. On the other hand control (without propolis) showed inferior properties after 7 days of storage and became unaccepted totally after 14 days where it was characterized by its pasty body, weak texture, gassiness and had a heavy surface yeast.

Table (3): Effect of addition of different levels of propolis on organoleptic properties of Labneh during storage period at 6°C.

Treatments		Storage period (weeks)	Organoleptic properties				
			Flavour 45	Body&Texture 30	Acidity 10	Appearance 15	Total 100
Control		Fresh	42^{a}	28^{ab}	9^{a}	15^{a}	94^{a}
		1	42^{a}	28^{ab}	9^{a}	15^{a}	94^{a}
		2	38^{b}	27^{b}	8^{b}	14^{ab}	87^{c}
		3	36^{c}	27^{b}	7^{c}	14^{ab}	84^{d}
Propolis concentration %	0.25	Fresh	40^{ab}	29^{a}	8^{b}	15^{a}	92^{b}
		1	41^{a}	28^{ab}	8^{b}	15^{a}	92^{b}
		2	40^{ab}	27^{b}	7^{c}	14^{ab}	88^{c}
		3	38^{b}	27^{b}	7^{c}	14^{ab}	86^{cd}
	0.50	Fresh	40^{ab}	28^{ab}	8^{b}	14^{ab}	91^{b}
		1	39^{b}	27^{b}	7^{c}	14^{ab}	87^{c}
		2	36^{c}	27^{b}	7^{c}	13^{b}	83^{de}
		3	36^{c}	26^{bc}	6^{d}	13^{b}	81^{e}
	0.75	Fresh	38^{b}	27^{b}	7^{c}	13^{b}	85^{d}
		1	36^{c}	26^{bc}	7^{c}	12^{bc}	81^{e}
		2	36^{c}	25^{c}	6^{d}	12^{bc}	79^{f}
		3	35^{d}	24^{d}	5^{e}	11^{c}	75^{g}
	1.00	Fresh	37^{bc}	26^{bc}	7^{c}	12^{bc}	82^{e}
		1	36^{c}	26^{bc}	6^{d}	12^{bc}	80^{ef}
		2	35^{d}	23^{e}	5^{e}	11^{c}	74^{g}
		3	33^{e}	22^{f}	5^{e}	10^{d}	70^{h}
L.S.D.			1.5271	1.6141	1.6024	1.0541	2.4213

- Different letters (a,b,c,…) means that multi comparisons are different from eash other, letter a is highest mean followed by b,c,…. Etc. Significant at 0.05 level

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تاثير صمغ عسل النحل على خمائر اللبنة وعلى جودة اللبنة المصنعة من اللبن الجاموسى

ايهاب عبد الباقى يوسف عيسوى
معهد بحوث تكنولوجيا الاغذية – مركز البحوث الزراعية

اختبر مدى فاعلية اربعة تركيزات من صمغ نحل العسل (البروبليز) على ثلاثة انواع من الخمائر التى تسبب فساد للبنة وهذة الخمائر هى:
Saccharomyces cerevisia, Debaryomyces hansenii and *Kluyveromyces marxianus.*
واستخدم مستخلص صمغ نحل العسل (١٠% وزن / حجم) بتركيزات ٢٥و% ، ٥٠و% ، ٧٥و% ، ١%.

ولوحظ التغير فى اعداد الخمائر بعد ٧ ايام من التحضين على ٢٥°م. واتضح من النتائج ان استخدام مستخلص صمغ النحل بنسبة ١% كان لها اثر فعال فى تثبيط نمو الخمائر على البيئة الصناعية, وان استخدام تركيز ٢٥و% كان لة تاثير ضعيف على تثبيط نمو الخمائر.

وتم تصنيع اللبنة باستخدام لبن جاموسى (٥و٥% دهن و٢% بادىء مختلط حيث تم تصنيع معاملة بدون اضافة (كنترول), واربعة معاملات باستخدام التركيزات السابقة من مستخلص صمغ النحل. وتم تحليل اللبنة كيميائيا وبكتيريولوجيا وحسيا على فترات ٠ , ١ , ٢ , ٣ اسابيع.

وأتضح من النتائج أنة كانت هناك اختلافات طفيفة في التركيب الكيماوى (الجوامد الكلية – الدهن – البرتين) للعينات ولكن أتضح أن المعاملات المضاف إليها مستخلص صمغ النحل انخفض محتواها من الحموضة والاسيتالدهيد والداى استيل عن عينة الكنترول وازداد هذا الانخفاض بزيادة تركيز مستخلص نحل العسل وكان هذا واضحاً أثناء التخزين في الثلاجة.

وعموماً فان الاختلافات بين المعاملات أثناء مدة التخزين كانت طفيفة في التركيب الكيماوى ولكن ارتفعت الحموضة والاسيتالدهيد والداى استيل في الكنترول أثناء التخزين حتى٣ اسابيع بعكس المعاملات التي تم إضافة مستخلص صمغ النحل إليها.

ارتفاع تركيز مستخلص صمغ النحل كان له أثراً واضحاً على الأعداد الميكروبية لكل من المحتوى الكلى للفطريات والخمائر والعدد الكلى للبكتريا وكذلك بكتريا حمض اللاكتيك.

استخدام ١% من البروبليز فى تصنيع اللبنة أعطى طعم ولون غير مقبولين, ولكن استخدام ٥و% من البروبليز فى تصنيع اللبنة كان ليس لة تأثيرا على لون وطعم اللبنة بالاضافة الى تاثيرة الفعال فى زيادة قوة حفظ اللبنة.